Structure-activity Relationships within a Series of C(7)-Substitutedoxyiminocephalosporins Containing the C(3)-Methylaminopyridiniumthiomethyl Substituent

Synthesis and Biological Properties of BRL 57342 and Some Close Analogues

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(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)-2-[(*Z*)-[(*S*)-carboxy(3,4-dihydroxyphenyl)methyl]oxyimino]acetamido]-3-(1-methylaminopyridinium-4-thiomethyl)ceph-3-em-4-carboxylate sodium salt (BRL 57342, **1f**) combines excellent *in vitro* antibacterial potency against Gram-positive and Gram-negative bacteria, including *P. aeruginosa* and *Acinetobacter* spp., with excellent stability to extended spectrum β-lactamases. This potency is reflected in *in vivo* efficacy studies.

In a previous communication[1], we described a series of 7α-formamidocephalosporins, in which the novel 1-(substitutedamino)pyridinium-4-thiomethyl group was incorporated into the C(3) position of the cephalosporin ring. This gave a series of analogues, which were highly active against Gram-negative bacteria, particularly *Pseudomonas* spp., and demonstrated stability towards bacterial β-lactamases. However, in common with literature precedent[2,3], none of these 7α-substituted cephalosporins demonstrated equivalent potency against Gram positive bacteria.

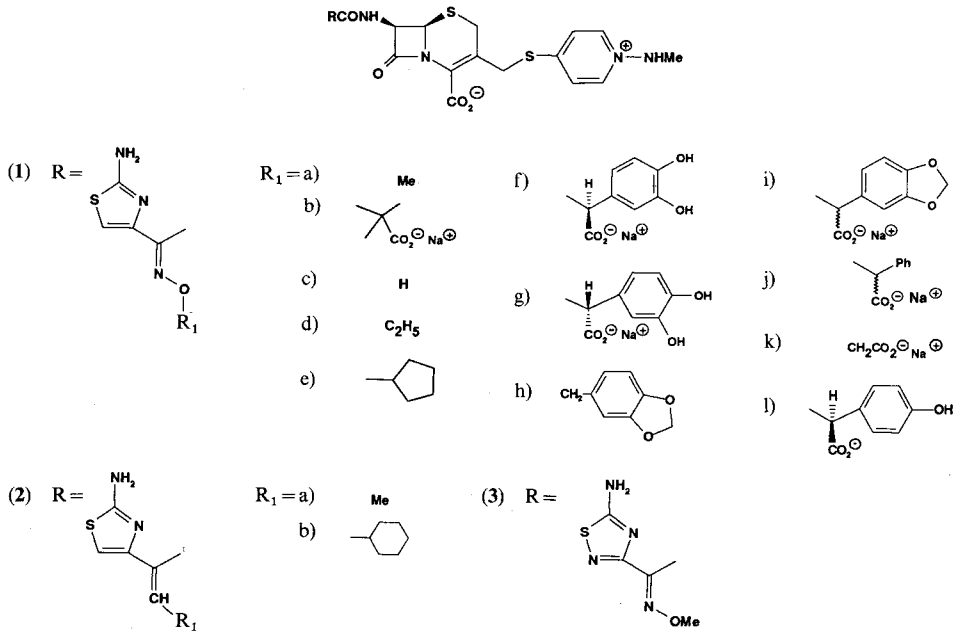
In contrast, the C(7)-methoxyiminoaminothiazolyl moiety is known to confer broad-spectrum potency when incorporated into a conventional cephalosporin nucleus[4] and some of these compounds are in clinical use. Accordingly, we embarked upon the synthesis of a series of C(7)-methoxyiminocephalosporins, designed to establish the optimal substitution of the C(3)-pyridiniumthiomethyl group. This approach led to the identification of BRL 55301 (**1a**) (Fig. 1)[5], a highly active cephalosporin demonstrating broad-spectrum antibacterial activity. However, **1a** demonstrated only a moderate level of activity against *Pseudomonas* spp. Our own work[1,3,6,7] and that of others[4,8~10], however, suggested that a number of structural modifications could be investigated with a view to improving the activity of this series of compounds against *Pseudomonas* spp. This approach culminated in the synthesis of BRL 57342 (**1f**), a C(7)-catechol-containing oxyiminocephalosporin with the desired enhancement in activity against *Pseudomonas* spp. Surprisingly, however, unlike other catechol-containing penicillins or cephalosporins[6], BRL 57342 (**1f**) still retains a clinically useful level of activity against Gram-positive bacteria. We now present our work on the synthesis and stucture-activity relationships of **1f** and some close analogues.

Chemistry

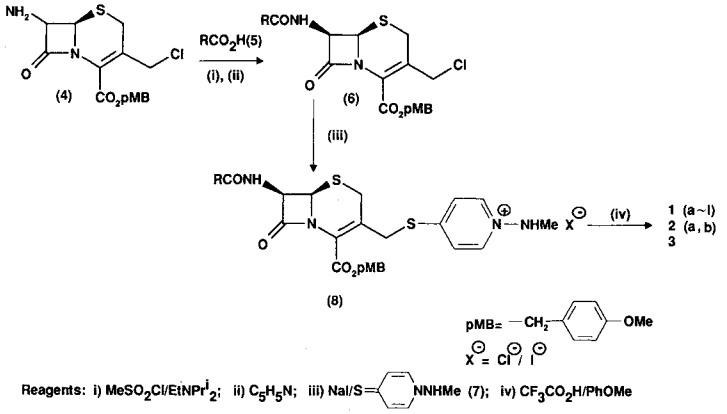
Compounds **1** (**a**~**l**), **2** (**a** and **b**) and **3** were prepared as shown in Scheme 1. The appropriately protected carboxylic acids (**5**) were converted to their mixed sulphonic anhydrides and condensed with cephalosporin nucleus (**4**) to yield intermediate esters (**6**) in variable yields. Reaction of C(3)-chloromethylcephems (**6**) with sodium iodide in acetonitrile, followed by 1-methylaminopyridin-4-thione (**7**)[11], gave the pyridinium salts (**8**) in high yields as a mixture of chloride and iodide salts. Treatment of the pyridinium salts (**8**) with trifluoroacetic acid and anisole in dichloromethane gave the betaines **1** (**a**~**l**), **2** (**a** and **b**) and **3**. Any hydroxyl groups present in side-chains (**5**) were protected as acetates, which were removed by hydrolysis at the final stage of the synthesis, and the amino groups of the thiazole and thiadiazole moieties were routinely protected with a trityl group. The carboxylic acids (**5**) were either commercially available or prepared by methods known in the literature[8,11,12].

An alternative route for the synthesis of BRL 57342 (**1f**) is shown in Scheme 2. The chloromethylcephem nucleus (**4**) was converted to pyridinium nucleus (**9**) by reaction with sodium iodide followed by pyridin-4-thione (**7**) and the product (**9**) isolated as a mixture of chloride and iodide salts[11]. Coupling of nucleus (**9**) with

Fig. 1.



Scheme 1. Synthesis of 3[-(1-methylamino)pyridinium-4-thiomethyl]cephalosporins (**1a**~**1l**), (**2a** and **2b**) and (**3**).



side-chain (**10**)[11], using a mixed sulphonic anhydride technique, gave the ester (**11**), again as a mixture of chloride and iodide salts. Trifluoroacetic acid treatment followed by aqueous hydrolysis at pH 10, successively removed the acid-labile protecting groups and the phenolic acetates to finally provide BRL 57342 (**1f**).

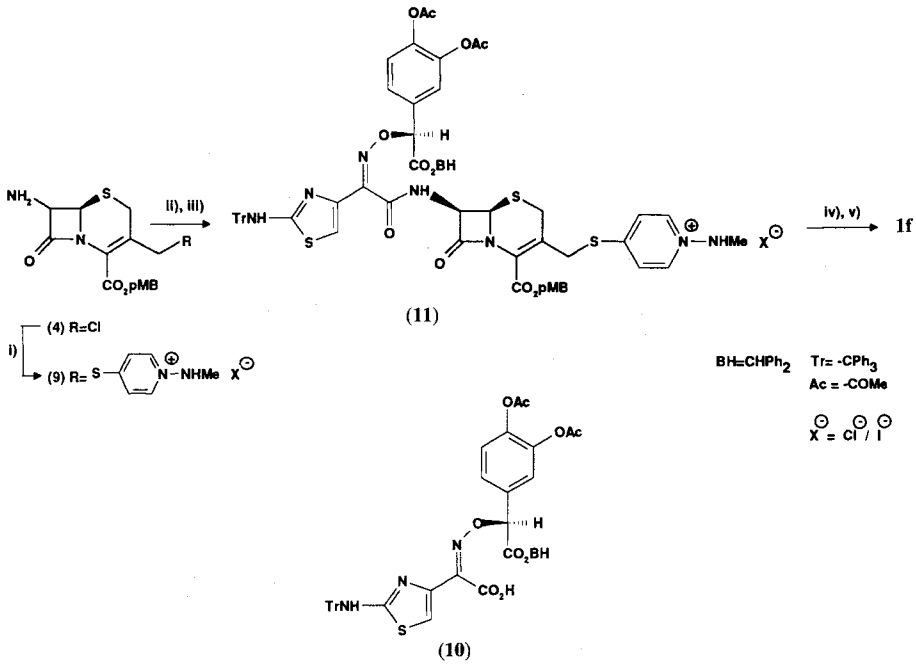
Results and Discussion

BRL 55301 (**1a**) exhibited good activity against *S. aureus* and most strains of *Enterobacteriaceae* but gave only moderate MICs against *P. aeruginosa* (Table 1). Replacement of the aminothiazolyl group by aminothiadiazolyl (**3**) had no obvious effect upon activity. Replacement of the methoxyimino group by ethylidene (**2a**) or cyclohexylmethylene (**2b**) groups caused loss of activity against Gram-negative organisms.

Further studies involved varying the substituent on the oxyimino group. The free oxime (**1c**) and the cyclopentyl derivative (**1e**) also demonstrated a loss of activity against Gram-negative bacteria and although the ethoxyimino analogue (**1d**) gave a 4~8 fold loss in Gram-positive activity, there were indications of improved potency against *P. aeruginosa*. Introduction of a carboxy group into the aliphatic oxyimino substituent (**1b** and **1k**) gave a further indication of enhanced activity against *P. aeruginosa*, but this was at the expense of reduced potency against *S. aureus* and, in the case of **1b**, against *Enterobacteriaceae* as well.

Introduction of a catechol group into the oxyimino substituent gave the diastereoisomers **1f** and **1g**. **1f** exhibited an exceptionally broad spectrum of activity,

Scheme 2. Synthesis of BRL 57342 (**1f**).



Reagents: i) (7)/NaI; ii) C_5H_5N; iii) (10)/$MeSO_2Cl$/$EtNPr^i_2$; iv) CF_3CO_2H/PhOMe; v) aq. $NaHCO_3$, pH10

Table 1. Antibacterial activity (MIC μg/ml*) of cephalosporin betaines.

Organism	1a	1b	1c	1d	1e	1f***	1g	1h	1i	1j	1k	1l**	2a	2b	3	CAZ	CFP
Escherichia coli DC0	<0.03	<0.03	0.5	<0.03	2	0.12	0.25	2	2	1	<0.03	0.12	0.12	2	<0.03	0.12	<0.03
E.coli DC0 R-TEM	<0.03	0.5	0.5	<0.03	4	0.12	0.5	2	2	1	<0.03	<0.06	0.06	2	<0.03	0.25	0.12
Klebsiella pneumoniae T767	<0.03	0.5	0.5	0.06	2	0.12	0.25	2	2	1	<0.03	—	0.12	4	<0.03	0.25	<0.03
Enterobacter cloacae N1	0.06	0.5	1	0.25	4	0.5	1	4	4	4	0.03	0.12	0.5	4	0.06	0.25	0.06
E. cloacae P99	4	32	4	4	8	2	8	8	>32	32	>32	>32	2	8	2	>32	1
Proteus mirabilis C 977	0.06	0.06	0.5	0.25	8	0.25	0.5	4	2	0.5	<0.03	—	0.25	8	0.25	0.12	0.06
Serratia marcescens US32	0.03	0.5	0.5	0.5	4	0.25	1	4	1	1	0.03	—	0.25	2	0.06	0.5	<0.03
Acinetobacter calcoaceticus W1G1	32	32	>32	>32	>32	2	>32	>32	>32	>32	32	—	64	>32	32	16	32
Pseudomonas aeruginosa K799WT	2	1	16	4	2	0.25	8	16	8	4	0.25	0.5	32	8	4	0.5	0.5
P. aeruginosa Dagleish	4	1	>32	1	16	1	16	8	4	4	1	1	32	16	4	1	1
P. aeruginosa Badia	>32	>32	>32	32	>32	1	16	32	16	16	>32	8	>64	32	>32	>32	32
Staphylococcus aureus Oxford	0.25	4	0.25	2	0.5	1	1	0.25	8	8	4	1	0.06	0.25	0.25	8	0.5
S. aureus Russell	0.5	4	0.5	2	—	1	1	0.25	8	8	4	1	0.25	0.5	1	8	0.5
Streptococcus pyogenes CN10	<0.03	<0.03	<0.03	<0.03	<0.03	0.12	0.12	<0.03	0.25	0.5	0.06	<0.06	<0.03	0.06	<0.03	0.12	<0.03

* Serial dilution in Isosensitest agar containing 5% defibrinated horse blood, inoculated with 0.001 ml of a 10^{-2} dilution of an overnight culture (10^4 cfu/spot).

** Tested in Mueller Hinton broth.

*** MIC figures for **1f** against the above test organisms in an iron-deficient medium showed a four-fold reduction clearly demonstrating the utilisation of the ton B-dependant iron transport mechanism[13~15]. **1l** showed no such effects.

CAZ: Ceftazidime.

CFP: Cefpirome.

which encompassed *P. aeruginosa* Badia and *Acinetobacter calcoaceticus* WIG1, with a maximum MIC of 2 μg/ml. This spectrum of activity was achieved at only slightly reduced activity, relative to **1a**, against *S. aureus* and *Enterobacteriaceae*. The *R* isomer (**1g**) was markedly less active than it's diastereoisomer (**1f**). The advantages conferred by the catechol group were clearly demonstrated by the lack of activity of the 3,4-methylenedioxyphenyl (**1i**) and phenyl (**1j**) derivatives, although simultaneous loss of the carboxylic acid group (**1h**) restored full activity against *S. aureus*. Replacement of the catechol by parahydroxyphenyl gave **1l**, which exhibited similar potency to **1f** against the majority of strains, but gave high MICs against *E. cloacae* P99 and *P. aeruginosa* Badia.

The broad spectrum activity of **1f** was confirmed in secondary tests (Table 2). Laboratory strains of *E. coli* K12, carrying plasmids mediating extended spectrum β-lactamases, generally exhibited markedly increased MICs against ceftazidime and cefpirome (Table 3).

Whilst elevated MICs were obtained for **1f** against some strains, the magnitude of these increases was generally small indicating improved β-lactamase stability over ceftazidime and cefpirome.

1f was *ca.* 30% bound in mouse, squirrel monkey and human serum. Unlike cefpirome and ceftazidime, **1f** exhibited prolonged and biphasic elimination in mice giving initial (α) and terminal (β) half lives of 0.34 and 1.2 hours (Table 4). In the mouse, the AUC for **1f** (23.4 μg·hour/ml) was similar to ceftazidime (30.1 μg·hour/ml) but *ca* half that of cefpirome (44.8 μg·hour/ml). In contrast, in the squirrel monkey, the AUC value for **1f** (34.6 μg·hour/ml) was higher than that of cefpirome (27.7 μg·hour/ml) but again similar to ceftazidime (34 μg·hour/ml). **1f** was highly efficaceous in mouse protection tests reflecting the *in vitro* activity (Table 5).

Table 2. Further *in vitro* evaluation of **1f**.

Species	No. Strains	MIC_{90} μg/ml*		
		1f	Cefpirome	Ceftazidime
S. aureus	38	0.25	2	16
E. coli	40	0.06	0.12	0.12
Enterobacter spp.	30	0.25	2	4
P. aeruginosa	80	4	16	8
Acinetobacter spp.	30	1	32	32

* Mueller Hinton agar.

Table 3. Activity of **1f** against *E. coli* J53 (K12 strain) producing extended spectrum β-lactamases.

β-lactamase	MIC_{90} μg/ml*		
	1f	Cefpirome	Ceftazidime
None**	0.03	0.03	0.06
TEM-3	0.5	2	16
TEM-4	0.25	2	8
TEM-5	0.25	0.12	4
TEM-7	0.12	2	16
TEM-9	0.5	4	>64
TEM-10	0.25	1	>64
TEM-E1	0.03	0.12	4
TEM-E2	0.12	2	32
TEM-E3	0.06	0.03	0.12
TEM-E4	0.12	1	16
SHV-2	0.12	1	2
SHV-3	0.03	0.25	0.5
SHV-5	1	4	>64
BIL-1	0.25	0.25	32
CAZ-3	0.06	0.12	1

* Mueller Hinton agar.
** Basal level of chromosomal enzyme.

Conclusion

As well as showing excellent Gram-negative activity, including *Pseudomonas* spp., BRL 57342 (**1f**) also demonstrates a surprising level of potency against *Staphylococcus* and *Streptococcus* spp. In addition, BRL 57342 (**1f**) exhibits good stability to bacterial β-lactamase enzymes, including extended spectrum β-lactamases, and excellent *in vivo* efficacy with an extended serum half-life in mice.

Experimental

IR spectra were recorded for dichloromethane solutions on a Perkin-Elmer 197 spectrophotometer or for KBr discs on Perkin-Elmer 457 or Perkin-Elmer 983 grating spectrophotometer. ^1H NMR spectra were obtained on Perkin-Elmer R32 (90 MHz) or Bruker WM

Table 4. Pharmacokinetics of **1f** in mouse and squirrel monkey.

Compound		Mouse (50 mg/kg; sc)		Squirrel Monkey (10 mg/kg; im)	
		$t_{1/2}$ (hours)	AUC ($0_{-\infty}$ μg·hour/ml)	$t_{1/2}$ (hours)	AUC ($0_{-\infty}$ μg·hour/ml)
1f	α)*	0.34	23.4	1.3	34.6
	β)	1.2			
Cefpirome		0.4	44.8	0.9	27.7
Ceftazidime		0.32	30.1	1.3	34

* Bi-phasic elimination; α) initial $t_{1/2}$, β) terminal $t_{1/2}$.

Table 5. *In vivo* efficacy of BRL 57342 in experimental mouse infections.

Test organism	Test	ED_{50}* (mg/kg)/(MIC μg/liter)**		
		1f	Cefpirome	Ceftazidime
Staphylococcus aureus Smith	ED_{50}	0.12	NT	7.5
	(MIC)	(0.5)		(4)
Escherichia coli JT415	ED_{50}	1.6	10	100
	(MIC)	(0.03)	(0.5)	(0.03)
Enterobacter cloacae T626	ED_{50}	5	1.2	>40
	(MIC)	(0.5)	(0.06)	(0.5)
Pseudomonas aeruginosa Horton	ED_{50}	14	480	100
	(MIC)	(0.5)	(4)	(2)

* Dosed at 1 and 5 hours post infection; *P. aeruginosa* dosed at 1, 3 and 5 hours.
** Mueller Hinton Agar.

250 (250 MHz) instruments using TMS or HOD as internal standard and the solvent is stated. Pyridinium salts (**8** and **11**) were isolated as mixtures of salts and produced complex ^{1}H NMR spectra. Only the major component is quoted. Fast atom bombardment mass spectra (FAB-MS) were recorded on a VG ZAB spectrometer and the matrix is quoted. All spectral data for the cephalosporin betaines (**1a**~**1l**) (**2a** and **2b**) and (**3**) are shown in Table 6.

Organic solutions were routinely dried over anhydrous magnesium sulphate and solvents were removed by evaporation under reduced pressure below 30°C. Preparative chromatography of cephalosporin esters was performed on silica gel (<230 mesh ASTM) (Merck) and of cephalosporin betaines on Diaion HP-20SS resin and the eluent is stated.

Compounds used for antibacterial testing were essentially single substances, analysed by reverse phase HPLC on a Gilson HPLC system. A Jones Chromatography Spherisorb S5 ODS2 column (30 cm × 4 mm i.d.), eluted with mixtures of acetonitrile and 0.05 M, pH 5.0, aqueous sodium acetate was used and compounds were

Table 6. Spectroscopic data for cephalosporins betaines (**1a**~**1l**), (**2a** and **2b**) and (**3**).

Compound	β-lactam carbonyl absorption, (KBr) cm^{-1}	^{1}H NMR spectra δ_H (250 MHz)	FAB *m/z* (matrix)
1a	1763	(D_2O) 3.01 (3H, s), 3.44, 3.72 (2H, ABq, J=14 Hz), 5.15 (1H, d, J=5 Hz), 5.74 (1H, d, J=5 Hz), 6.96 (1H, s), 7.78 (2H, d, J=7 Hz), 8.50 (2H, d, J=7 Hz).	MH^+ 536 (thioglycerol)
1b	1763	(D_2O) 1.44 (3H, s), 1.45 (3H, s), 3.00 (3H, s), 3.45, 3.70 (2H, ABq, J=18 Hz), 4.15, 4.38 (2H, ABq, J=14 Hz), 5.16 (1H, d, J=5 Hz). 5.75 (1H, d, J=5 Hz), 6.94 (1H, s), 7.80 (2H, d, J=7 Hz), 8.50 (2H, d, J=7 Hz).	MH^+ 630 (thioglycerol)
1c	1764	(D_2O) 3.01 (3H, s), 3.43, 3.71 (2H, ABq, J=18 Hz), 4.11, 4.39 (2H, ABq, J=14 Hz), 5.15 (1H, d, J=5 Hz), 5.78 (1H, d, J=5 Hz), 6.92 (1H, s), 7.78 (2H, d, J=7 Hz), 8.50 (2H, d, J=7 Hz).	MH^+ 522 (thioglycerol)
1d	1762	[D_2O+$(CD_3)_2CO$] 1.27 (3H, t, J=7 Hz), 3.02 (3H, s), 3.45, 3.71 (2H, ABq, J=18 Hz), 4.13, 4.42 (2H, ABq, J=14 Hz), 4.23 (2H, q, J=7 Hz), 5.16 (1H, d, J=5 Hz), 5.77 (1H, d, J=5 Hz), 6.94 (1H, s), 7.81 (2H, d, J=7 Hz), 8.53 (1H, d, J=7 Hz).	MH^+ 550 thioglycerol
1e	1762	(D_2O) 1.4~2.4 (8H, m), 3.00 (3H, s), 3.43, 3.69 (2H, ABq, J=18 Hz), 3.80~4.00 (1H, m), 4.11, 4.40 (2H, ABq, J=14 Hz), 5.13 (1H, s), 5.16 (1H, d, J=5 Hz), 5.71 (1H, d, J=5 Hz), 6.93 (1H, s), 7.78 (2H, d, J=8.0 Hz), 8.50 (2H, d, J=7 Hz)	M^+ 590 (thioglycerol/ acetic acid)
1f	1762	(D_2O) 3.01 (3H, s), 3.16, 3.49 (2H, ABq, J=18 Hz), 4.16, 4.30 (2H, ABq, J= 14 Hz), 5.01 (1H, d, J=5 Hz), 5.38 (1H, s), 5.62 (1H, d, J=5 Hz), 6.80~6.97 (4H, m), 7.80 (2H, d, J=7 Hz), 8.48 (2H, d, J=7 Hz)	MH^+ 710 (thioglycerol)
1g	1762	(D_2O) 2.99 (3H, s), 3.13, 3.45 (2H, ABq, J=18 Hz), 4.11, 4.35 (2H, ABq, J= 14 Hz), 4.96 (1H, d, J=5 Hz), 5.36 (1H, s), 5.59 (1H, d, J=5 Hz), 6.79~6.97 (4H, m), 7.76 (2H, d, J=7 Hz), 8.46 (2H, d, J=7 Hz)	MH^+ 710 (thioglycerol)
1h	1765	[$(CD_3)_2SO$] 2.99 (3H, br s), 3.20~3.50 (2H, m), 4.39, 4.59 (2H, ABq, J=14 Hz), 5.01 (3H, m), 5.57 (1H, dd, J=5, 8 Hz), 6.00 (2H, s), 6.71 (1H, s), 6.80~6.97 (3H, m), 7.23 (2H, br, exchangeable), 8.32 (2H, d, J=7 Hz), 8.43(2H, d, J=7 Hz), 8.45~8.70 (1H, br m, exchangeable), 8.81 (2H, d, J=7 Hz), 9.60 (1H, d, J=8 Hz, exchangeable)	MH^+ 656 (thioglycerol acetic acid)
1i	1762	(D_2O) 3.04, 3.06 (each 3H, 2s), 3.19~3.62 (2H, m), 4.21~4.36 (2H, m), 5.09 (1H, d, J=5 Hz), 5.47 (1H, s), 5.68, 5.70 (each 1H, 2d, J=5 Hz), 5.99 (2H, br s), 6.80~7.10 (4H, m), 7.87, 7.90 (each 2H, 2d), 8.34 (2H, d, J=7 Hz)	[M^+] 721 (thioglycerol)
1j	1762	(D_2O) 2.99 (3H, br s), 3.13~3.54 (each 2H, 2ABq, J=18 Hz), 4.12~4.32 (2H, m), 5.01, 5.03 (each 1H, 2d, J=5 Hz), 5.51 (1H, br s), 5.62, 5.64 (each 1H, 2d, J=5 Hz), 6.96, 6.97 (each 1H, 2s), 7.28~7.55 (5H, m), 7.78~7.84 (2H, m), 8.42~8.54 (2H, br m)	[MH^+] 656 (thioglycerol)
1k	1762	(D_2O) 3.00 (3H, s), 3.44, 3.69 (2H, ABq, J=18 Hz), 4.17, 4.36 (2H, ABq, J=14 Hz), 4.52 (2H, s), 5.15 (1H, d, J=5 Hz), 5.75 (1H, d, J=5 Hz), 6.98 (1H, s), 7.82 (2H, d, J=7 Hz), 8.50 (2H, d, J=7 Hz)	[MH^+] 602 (thioglycerol)
1l	1768	[$(CD_3)_2SO$] 2.89 (3H, s), 3.25, 3.45 (2H, ABq, J=17 Hz), 4.13, 4.67 (2H, ABq, J=13 Hz), 5.06 (1H, d, J=5 Hz), 5.24 (1H, s), 5.72 (1H, dd, J=5, 8 Hz), 6.70 (2H, d, J=8 Hz), 6.77 (1H, s), 7.22 (2H, br s, exchangeable), 7.33 (2H, d, J=8 Hz), 8.20 (2H, d, J=7 Hz), 8.68 (2H, d, J=7 Hz), 11.10 (1H, br s, exchangeable)	[MH^+ − Na] 672 (thioglycerol)
2a	1762	(D_2O) 1.2~2.4 (11H, m), 3.12 (3H, s), 3.56, 3.82 (2H, ABq, J=18 Hz), 4.17, 4.52 (2H, ABq, J=14 Hz), 5.26 (1H, d, J=5 Hz), 5.82 (1H, d, J=5 Hz), 6.26 (1H, d, J=11 Hz), 6.55 (1H, s), 7.90 (2H, d, J=7 Hz), 8.61 (2H, d, J=7 Hz)	[MH^+] 639 (thioglycerol)
2b	1762	(D_2O) 1.82 (3H, d, J=7 Hz), 2.99 (3H, s), 3.40, 3.69 (2H, ABq, J=18 Hz), 4.06, 4.40 (2H, ABq, J=14 Hz), 5.13 (1H, d, J=5 Hz), 5.73 (1H, d, J=5 Hz), 6.38 (1H, q, J=7 Hz), 6.44 (1H, s), 7.76 (2H, d, J=7 Hz), 8.49 (2H, d, J=7 Hz)	MH^+ 587 (thioglycerol)
3	1762	[D_2O+$(CD_3)_2CO$] 3.16 (3H, s), 3.53, 3.79 (2H, ABq, J=18 Hz), 4.13 (3H, s), 4.31, 4.59 (2H, ABq, J=14 Hz), 5.23 (1H, d, J=5 Hz), 5.90 (1H, d, J=5 Hz), 8.00 (2H, d, J=7 Hz), 8.71 (2H, d, J=7 Hz)	MH^+ 537 (thioglycerol)

detected by UV absorption at 254 nm.

General Procedure for Preparation of Cephalosporin betaines (**1a**~**1l**), (**2a** and **2b**) and (**3**)

Sodium [6*R*,7*R*]-7-[2-(2-Amino-4-thiazolyl)-2-[(*Z*)-[(*R*,*S*)-carboxyphenylmethyl]oxyimino]acetamido]-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate (**1j**)

a) 4-Methoxybenzyl [6*R*,7*R*]-3-chloromethyl-7-[[(*Z*)-[(*R*,*S*)-diphenylmethoxycarbonylphenylmethyl]-oxyimino]-(2-tritylamino-4-thiazolyl)acetamido]ceph-3-em-4-carboxylate (**6**)

Z-[(*R*,*S*)-Diphenylmethoxycarbonylphenylmethyl]-oxyimino-(2-tritylamino-4-thiazolyl)acetic acid (**5**)[11] (303 mg; 0.4 mmol) in dry dichloromethane (10 ml) under argon, was treated with diisopropylethylamine (54 mg; 0.4 mmol) followed by methanesulphonyl chloride (48 mg; 0.4 mmol) and the mixture heated under reflux until IR analysis showed that little or no starting acid remained. The reaction mixture was cooled to room temperature and treated with a mixture of 4-methoxybenzyl [6*R*,7*R*]-7-amino-3-chloromethylceph-3-em-4-carboxylate (**4**) (153 mg; 0.4 mmol) and pyridine (33 mg; 0.4 mmol) in dry dichloromethane (10 ml). The reaction mixture was stirred at room temperature for 1 hour then evaporated to dryness under reduced pressure. The residue was treated with ethyl acetate (30 ml) and water (30 ml) and the phases separated. The aqueous phase was further extracted with ethyl acetate (20 ml) and the extracts combined, washed with water, saturated brine, dried and evaporated to dryness. The resulting foam was purified by chromatography, eluting with mixtures of ethyl acetate and hexane, to give the title compound as an off-white foam (230 mg; 51%): IR (CH_2Cl_2) cm^{-1} 1790, 1730; ^1H NMR (250 MHz; $CDCl_3$) 3.12 and 3.48 and 3.38 and 3.56 (each 2H, 2×ABq, J=18 Hz, isomers), 3.82 (3H, s), 4.39~4.63 (2H, m), 4.94 and 4.98 (each 1H, 2d, J=5 Hz, isomers), 5.29~5.38 (3H, m), 5.83~5.92 (1H, m, isomers), 6.78~7.44 (37H, m); FAB-MS (3-nitrobenzyl alcohol-sodium acetate) m/z 1102 (M+Na, $C_{61}H_{50}ClN_5O_8S_2Na$).

b) 4-Methoxybenzyl [6*R*,7*R*]-7-[[(*Z*)-[(*R*,*S*)-diphenylmethoxycarbonylphenylmethyl]oxyimino]-(2-tritylamino-4-thiazolyl)acetamido]-3-[(1-methylamino)-pyridinium-4-thiomethyl)]ceph-3-em-4-carboxylate chloride/iodide salt (**8**)

The product from a) (230 mg; 0.21 mmol) was dissolved in acetonitrile (2 ml) and treated with 1-methylaminopyridin-4-thione (**7**)[11] (29.8 mg; 0.21 mmol) and sodium iodide (32 mg; 0.21 mmol). The mixture was stirred at room temperature for 1 hour then evaporated to dryness under reduced pressure. The residual, yellow foam was purified by chromatography, eluting with mixtures of methanol and dichloromethane, to give the title compound as a yellow, amorphous solid (158 mg; 57%): IR (CH_2Cl_2) cm^{-1} 1790, 1730; 1H NMR (250 MHz; $CDCl_3$) 3.03 and 3.06 (each 3H, 2d, J=6 Hz, isomers), 3.17 and 3.49 and 3.40 and 3.58 (each 2H, 2×ABq, J=18 Hz, isomers), 3.81 (each 3H, 2×s, isomers), 4.23~4.42 (2H, m), 4.98 and 5.01 (each 1H, 2×d, J=5 Hz, isomers), 5.11~5.28 (2H, m, isomers), 5.80~5.90 (1H, m, isomers), 6.08~6.11 (each 1H, 2×s, isomers), 6.82~7.54 (36H, m), 8.06 and 8.35 (each 1H, d, J=7 Hz, exchangeable, isomers), 7.58~7.64 and 8.64~8.78 (each 2H, 2×m, isomers), 8.91 (1H, q, J=6 Hz, exchangeable); FAB-MS (thioglycerol) m/z 1185 (M+H, $C_{67}H_{59}IN_7O_8S_3$).

c) Sodium [6*R*,7*R*]-7-[(2-amino-4-thiazolyl)-[(*Z*)-[(*R*,*S*)-carboxyphenylmethyl]oxyimino]acetamido]-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate (**1j**)

The product from b) (159 mg; 0.12 mmol) was dissolved in dry dichloromethane (5 ml) and treated with trifluoroacetic acid (1.24 g; 11 mmol). After 1 hour, the volatiles were removed under reduced pressure and the residue treated with water and acetone and the pH of the mixture adjusted to 7.5 with saturated, aqueous sodium hydrogencarbonate solution. The mixture was filtered, diluted with ethyl acetate (50 ml), the phases separated and the aqueous phase freeze-dried. Dissolution of the crude, freeze-dried product in water (minimum volume) followed by chromatography, eluting with mixtures of tetrahydrofuran in water, gave the title compound as a yellow solid after freeze-drying (20 mg; 24%): IR (KBr) cm^{-1} 1762; ^1H NMR (250 MHz; D_2O) 2.99 (3H, br s) 3.13~3.54 (each 2H, 2×ABq J=18 Hz isomers) 4.12~4.32 (2H, m, isomers), 5.01 and 5.03 (each 1H, 2×d, J=5 Hz, isomers), 5.5 (1H, br s), 5.62 and 5.64 (each 1H, 2×d, J=5 Hz, isomers), 6.96 and 6.97 (each 1H, 2s, isomers), 7.28~7.55 (5H, m), 7.78~7.84 and 8.42~8.54 (each 2H, 2×m, isomers); FAB-MS (thioglycerol) m/z 678 (M+H, $C_{27}H_{25}N_7O_7S_3Na$).

Synthesis of Sodium [6*R*,7*R*]-7-[(2-Amino-4-thiazolyl)-[(*Z*)-[(*S*)-carboxy(3,4-dihydroxyphenyl)methyl]-oxyimino]acetamido]-3-[(1-methylamino)pyridinium-4-thiomethyl)]ceph-3-em-4-caboxylate (**1f**)

a) 4-Methoxybenzyl [6*R*,7*R*]-7-amino-3-[1-(methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate chloride/iodide salt (**9**)

4-Methoxybenzyl [6*R*,7*R*]-7-amino-3-(chloromethyl)-ceph-3-em-4-carboxylate hydrochloride (**4**) (2.4 g; 6 mmol) was partitioned between dilute, aqueous sodium hydrogen carbonate (50 ml) and ethyl acetate (50 ml). The organic phase was washed with saturated brine, dried and evaporated to dryness. The residue was dissolved in acetonitrile (20 ml) and treated with sodium iodide (0.75 g; 5 mmol). After 5 minutes, **7** (0.7 g; 5 mmol) was added and the mixture stirred for 1.5 hours. The mixture was filtered into vigorously-stirred diethyl ether (500 ml) and the product collected by filtration (2.2 g; 72%); IR (KBr) cm^{-1} 1772, 1718, 1618, 1513; ^1H NMR (250 MHz; $CDCl_3$) 3.07 (3H, d, J=6 Hz), 3.49 and 3.73 (2H, ABq, J=18 Hz), 3.81 (3H, s), 4.29 and 4.45 (2H,

ABq, $J=13$ Hz), 4.79 (1H, d, $J=5$ Hz), 4.97 (1H, d, $J=5$ Hz), 5.20 and 5.28 (2H, ABq, $J=12$ Hz), 6.87 (2H, d, $J=8.5$ Hz), 7.36 (2H, d, $J=8.5$ Hz), 7.73 (2H, d, $J=7$ Hz), 8.51 (1H, q, $J=6$ Hz, exchangeable), 8.89 (2H, d, $J=7$ Hz); FAB-MS (thioglycerol) m/z 473 (M^+, $C_{22}H_{25}N_4O_4S_2$).

b) 4-Methoxybenzyl [6*R*,7*R*]-7-[[(*Z*)-[(*S*)-(3,4-diacetoxyphenyl)-diphenylmethoxycarbonylmethyl]oxyimino]-(2-tritylamino-4-thiazolyl)acetamido]-3-[(1-methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate chloride/iodide salt (**11**)

Compound **10**[11] (845 mg; 1 mmol) was dissolved in dry DMF (2 ml) under argon and the solution cooled to 0°C. Diisopropylethylamine (129 mg; 1 mmol) was added and the mixture cooled further to −40°C. Methanesulphonyl chloride (114 mg; 1 mmol) was added and the mixture stirred at −20°C for 0.5 hours. A solution of (**9**) (600 mg) and pyridine (79 mg; 1 mmol) in dry dichloromethane (5 ml) was added at −40°C and cooling removed. The mixture was stirred at ambient temperatures for 1 hour then diluted with dichloromethane (20ml). The mixture was washed with water (5×), dried and evaporated to dryness. The residue was purified by chromatography, eluting with mixtures of methanol in dichloromethane, to give the title compound (**11**) as an amorphous solid (271 mg; 19%): IR (KBr) cm^{-1} 1774, 1730; ^1H NMR [250 MHz; $(CD_3)_2SO$] 2.23~2.28 (6H, br s), 3.00 (3H, d, $J=6$ Hz), 3.71 (3H, s), 4.27 (2H, br s), 5.12 (1H, d, $J=5$ Hz), 5.20 (2H, br s), 5.52~5.58 (1H, dd, $J=5$ and 8 Hz), 5.87 (1H, s), 6.78~6.89 (4H, m), 7.14~7.48 (20H, m), 7.88 (2H, d, $J=7$ Hz), 8.16 (1H, q, $J=6$ Hz, exchangeable), 8.76 (2H, d, $J=7$ Hz), 8.96 (1H, br s), 9.62 (1H, d, $J=8$ Hz, exchangeable); FAB-MS (thioglycerol) m/z 1300 (M^+, $C_{71}H_{62}N_7O_{12}S_3$).

c) Sodium [6*R*,7*R*]-7-[(2-amino-4-thiazolyl)-[(*Z*)-[(*S*)-carboxy(3,4-dihydroxyphenyl)methyl]oxyimino]-acetamido]-3-[(1-methylamino)pyridinium-4-thiomethyl]ceph-3-em-4-carboxylate (**1f**).

The fully-protected cephem (**11**) (250 mg; 0,18 mmol) was dissolved in dichloromethane (15 ml) and treated with trifluoroacetic acid (906 mg; 7.9 mmol) at room temperature. When TLC analysis showed little or no starting material remaining, the volatiles were removed by evaporation under reduced pressure and the residue evaporated from toluene (2×). The residue was dissolved in methanol (minimum volume), diluted with water and the pH of the mixture adjusted to 10.5 with saturated, aqueous sodium hydrogen carbonate. When HPLC analysis showed no diacetate remaining, the volatiles were removed under reduced pressure and the residue freeze-dried. Purification by chromatography, eluting with mixtures of tetrahydrofuran in water, gave the product (**1f**) after freeze-drying (30 mg; 24%): IR (KBr) cm^{-1} 1762; ^1H NMR (250 MHz; D_2O) 3.01 (3H, s), 3.16 and 3.49 (2H, ABq, $J=18$ Hz), 4.16 and 4.30 (2H, ABq, $J=14$ Hz), 5.01 (1H, d, $J=5$ Hz), 5.38 (1H, s), 5.62 (1H, d, $J=5$ Hz), 6.80~6.97 (4H, m), 7.80 and 8.48 (each 2H, 2×d, $J=7$ Hz); FAB-MS (thioglycerol) m/z 710 (M+H, $C_{27}H_{25}N_7O_9S_3Na$).

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